

# Skin RT-QuIC Assays are More Sensitive than CSF RT-QuIC in Prion Detection for Chinese Probable Sporadic Creutzfeldt-Jakob Disease

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## Research

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# Abstract

## Background

The definite diagnosis of human sporadic Creutzfeldt-Jakob disease (sCJD) largely depends on postmortem neuropathology and PrP<sup>Sc</sup> detection in the brain. The development of prion RT-QuIC of cerebrospinal fluid (CSF) samples makes it possible for premortem diagnosis for sCJD. However the diagnostic potential of RT-QuIC of skin specimen for probable sCJD is not well researched. This study is to evaluate the diagnostic potential of RT-QuIC of skin specimen in human prion diseases.

## Methods

We collected the paired skin and CSF samples from 29 recruited alive patients referred to Chinese CJD surveillance center, including 12 probable sCJD, 9 non-CJD, 3 genetic prion disease (gPrD) and 5 cases whose diagnoses still pending. The samples were subjected to RT-QuIC assays using recombinant hamster PrP protein rHaPrP90-231 as the substrate.

## Results

All 12 probable sCJD patients, 4 pending, and 1 T188K genetic CJD (gCJD) cases showed positive prion-seeding activity, while all 9 non-CJD patients were negative. CSF RT-QuIC positive seeding activity was only observed in 5 probable sCJD patients.

## Conclusions

Our preliminary data indicate high sensitivity and specificity of skin RT-QuIC in prion detection for Chinese probable sCJD and highlight that skin prion-seeding activity is a reliable biomarker for premortem diagnosis of human prion disease.

## Background

Human prion disease (PrD) is a group of transmissible neurodegenerative diseases, consisting of sporadic form, e.g., sporadic Creutzfeldt-Jakob disease (sCJD), genetic or familial form, e.g, genetic CJD (gCJD), fatal familial insomnia (FFI), Gerstmann-Sträussler-Scheinker disease (GSS), and acquired form, e. g., iatrogenic CJD (iCJD) and variant CJD (vCJD) [1-3]. sCJD is the commonest type of human PrD with the incidence of 1 to 2 patient per million per year. The definite diagnosis of human PrD, particularly for sCJD, is still largely dependent on the postmortem brain tissues showing special neuropathological changes, i.e., spongiform degeneration, and/or PrP<sup>Sc</sup> deposit [2], although some biomarkers in cerebrospinal fluid (CSF) have revealed significances in diagnosis of sCJD, such as 14-3-3 and tau [4].

In the past decade, the diagnostic predicament for CJD has been greatly improved due to the development of RT-QuIC assay [5]. The implementation of RT-QuIC in detection of prions in CSF samples has been verified the meaningful diagnostic value for sCJD clinically [5, 6] . Some countries, such as USA,

UK and Japan, have even listed the CSF RT-QuIC test in the national diagnostic standard or criterion for sCJD [7, 8]. However, the accuracies of CSF RT-QuIC assays in sCJD diagnosis may vary among the different laboratories, ranging from 60% to almost 100% [5, 9, 10]. On the other hand, collecting CSF sample through spinal tap is an invasive process in clinic, and some patients fail to provide CSF due to contraindications or refusal to lumbar puncture. The feasibility of usage of RT-QuIC in other easily-obtained specimen is mostly desired.

Recently, the detection of prions in skin specimen with RT-QuIC test has been reported by Zou's team. They have found that prions could be detected from the autopsy skin tissues of sCJD and vCJD patients [11]. Based on the animal experiments, they have also proved that prions could be detected in skin tissues even before the onset of clinical symptoms [12]. These results highlight a good applied prospect of skin RT-QuIC in the premortem diagnosis of human PrDs. In this study, we obtained the paired skin and CSF samples from 29 recruited alive patients referred to Chinese CJD surveillance center, including 12 probable sCJD and 9 non-CJD. Positive reaction in skin RT-QuIC assay was observed in all 12 sCJD cases, but not in non-CJD cases. Meanwhile, only 5 out of 12 probable sCJD were positive in CSF RT-QuIC tests. Our results indicated that skin specimen was ideal for RT-QuIC test in Chinese patients.

## Materials And Methods

### *Samples*

The paired skin and CSF samples from 29 patients with probable sCJD, gPrD or non-CJD were obtained from the tissue bank in the Center of Chinese CJD Surveillance System, including 12 sCJD, 1 T188K gCJD, 1 G114V gCJD, 1 D178N FFI, 9 non-CJD and 5 pending CJD cases. Among them, 4 patients provided two skin samples collected from different sites. The demography information of the patients, the clinical data, MRI and EEG data, the results of the Western blot for CSF 14-3-3 and *PRNP* sequencing were collected from the database of the Center of Chinese CJD Surveillance System.

In addition, 10% skin homogenates of neuropathologically-confirmed 10 sCJD patients and 5 non-CJD controls from the National Prion Disease Pathology Surveillance Center (NPDPSC), Case Western Reserve University School of Medicine, Cleveland, Ohio, USA, were also enrolled in this study for validation of our skin RT-QuIC assay. All 5 non-CJD subjects were determined by neuropathological assays with the postmortem brains, which excluded the possibilities of sCJD or other human prion diseases but did not have the diagnosis for other neurological diseases.

All enrolled CSF samples were obtained by lumbar puncture and were free of blood contamination. Routine CSF biochemistry assays of those specimens, including cell count, glucose and total protein were all in the normal ranges.

### *Preparation of homogenates of skin*

The sites for skin biopsies in this study included the skins behind the ears, inside the arms, inside the thighs, lower back, and/or abdomen. After disinfection with 75% alcohol, the patient received local anesthesia with subcutaneous injection of 1-2% lidocaine hydrochloride. A small piece of skin with a size of about 0.2 x 0.3 cm<sup>2</sup> was taken with a disposable skin biopsy punch (Acupunch, Acuderm Inc, US), according to the manufacturer's instruction. Usually, the biopsy skin specimen covers epidermis, dermis, and adipose tissues. 2% (w/v) of skin homogenate was prepared in lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mM Tris, pH7.5) according to a previously described protocol[11, 12]. The homogenates were then stored at -80 ° C for further used.

### *RT-QuIC assays*

The detail procedures of the RT-QuIC assay was described previously[13]. Briefly RT-QuIC reaction contained 10 µg of rHaPrP90-231, 1X PBS, 170 mM NaCl, 1 mM EDTA, 0.01 mM ThT, 0.001% SDS, together with 15 µl CSF samples or 2 µl 10<sup>-2</sup> to 10<sup>-4</sup> diluted skin homogenates in a final volume of 100 µl. Each sample was assayed in triplicated or quadruplicated. The assay was conducted in a black 96-well, optical-bottomed plate (Nunc, 265301) on a BMG FLUOstar plate reader (BMG LABTECH). The main working conditions were fixed as follow: temperature, 55°C; vibration speed, 700 rpm; vibration/incubation time, 60/60 sec; total reaction time, 60 h. ThT fluorescence (excitation wavelength, 450 nm; emission wavelength, 480nm) each reaction was automatically measured every 45 min and expressed as relative fluorescence units (rfu). The cutoff value was set as the mean value of the negative controls plus 10 times the standard deviation. A sample was considered to be positive when ≥2 wells revealed positive reaction curves. The positive control was 10<sup>-5</sup> diluted the brain homogenate of the scrapie agent 263K-infected hamster, while the negative control was 10<sup>-5</sup> diluted the brain homogenate of normal hamster.

### *Ethics approval*

Usages of the CSF and skin samples and relevant clinical information of the patients with different diseases in the Center of Chinese CJD Surveillance System has been approved by the Ethics Committee of the National Institute for Viral Disease Control and Prevention, China CDC, under the protocol of 2009ZX10004-101.

## **Results**

Our RT-QuIC assay for CSF samples had been blindly evaluated previously with a sensitivity of 96.7% and a specificity of 100% by CSF samples of neuropathologically-confirmed 30 definite sCJD and 30 non-CJD provided kindly by the NPDPSC (paper in preparation). In this study, the skin samples of the coded 10 definite sCJD patients and 5 non-CJD controls from NPDPSC with a dilution from 10<sup>-2</sup> to 10<sup>-4</sup> were examined blindly by our RT-QuIC assay. The main demographic information of these 15 subjects was summarized in Table 1. RT-QuIC assays revealed the positive reactions in all definite sCJD samples and negative reactions in all non-CJD samples (Table 1), indicating 100% sensitivity and specificity of our RT-QuIC assay for prion detection in the skin samples of sCJD and controls.

The paired skin and CSF samples from 29 patients with different clinical diagnoses were enrolled into RT-QuIC assays in this study. The main demographic, clinical examination and laboratory information of the 29 patients were summarized in Table 2. The median of the onset age of 12 probable sCJD cases was 67 years (y) old (ranging from 54 to 84 y), while that of 9 non-CJD cases was 65 y (43-83 y). Majority of the patients were MM homozygous in codon 129 and EE homozygous in codon 219 of prion protein. Periodic sharp wave complexes (PSWC) on EEG were recorded in 5 of 12 sCJD cases and the T188K gCJD case, but not in 5 non-CJD cases who undertook the examination of EEG. sCJD associated abnormalities (high signal in caudate/putamen and/or symmetrical or dissymmetrical cortical ribbon syndrome on diffusion-weighted imaging (DWI)) were noticed in 8 out of 11 sCJD patients, 4 out of 5 pending cases, T188K and G114V gCJD cases, as well as 5 out 7 non-CJD cases, particularly the cortical ribbon syndrome. CSF 14-3-3 protein showed positive in 7 out 11 sCJD cases, but also in 3 out 9 non-CJD patients.

Aliquot of 15  $\mu$ l CSF sample from each patient was subjected into RT-QuIC tests. Only 5 sCJD cases, the T188K gCJD case and 2 pending cases revealed positive reactions, while the rest of the tested CSF samples was negative (Table 2, Fig 1). Differently diluted skin homogenates from 24 patients were also employed into RT-QuIC assays. As shown in Table 2 and Fig 1, all 12 sCJD cases were positive, whereas all 9 non-CJD cases were negative. Additionally, the T188K gCJD cases and 4 out 5 pending cases showed also positive in RT-QuIC tests, while the G114V gCJD and D178N FFI cases showed negative. Those data highlight that RT-QuIC reactivity or sensitivity of skin sample of sCJD patients is higher than that of CSF sample.

Further, the RT-QuIC reactivity of each positive skin sample was analyzed based on the dilution of the skin homogenate and the reactivity in every testing well. As shown in Table 3, the general positive rates of the tested skin samples revealed a notable dose-dependent pattern, showing 90.5% (19/21) positive rate in the reaction of  $10^{-2}$  dilution, 66.7% (14/21) in that of  $10^{-3}$  and 14.3% (3/21) in that of  $10^{-4}$  dilution. The RT-QuIC positive reactivity of the most tested samples reduced along with the increase of dilution, with two exceptions. One showed positive reaction in the preparation of  $10^{-3}$ , but negative in those of  $10^{-2}$  and  $10^{-4}$ , while the other was positive in the reaction of  $10^{-4}$ , but negative in that of  $10^{-2}$  and  $10^{-3}$ . It seems that the skin samples of human prion diseases possess the similar dose-dependent RT-QuIC reactive profile as the brain samples.

Four patients (sCJD Cases 9 and 12, as well as pending cases 4 and 5) donated two skin samples simultaneously, collected from different sites including posterior neck, lateral malleolus, medial forearm, behind ear and inner thigh. Although all samples from those 4 cases were positive in RT-QuIC tests, the reactivity was slightly different. The samples of posterior neck in the sCJD Case 9 and pending Case 5 were positive in the reactions of all dilutions ( $10^{-2}$  to  $10^{-4}$ ), while the samples of lateral malleolus from the same patients elicited positive RT-QuIC reactions only in the dilutions of  $10^{-2}$  and  $10^{-3}$  (Table 3). Analyses of the RT-QuIC reactive curves of sCJD Case 9 (Fig 2A) and pending Case 5 (2B) identified more higher ThT values in the samples of back neck than that of lateral malleolus. sCJD Case 12 donated the skin samples from back neck and medial forearm. Both samples induced positive RT-QuIC reactions at the

dilution of  $10^{-3}$ , although one well in the preparation of back neck showed positive curve at the dilution of  $10^{-4}$  (Table 3). Pending Case 4 donated the samples of back ear and inner thigh. Positive RT-QuIC was observed in the sample of inner thigh at the dilution of  $10^{-3}$ , but only in back ear at the dilution  $10^{-2}$  (Table 3). RT-QuIC reactive curves of sCJD Case 12 (Fig 2C) and pending Case 4 (Fig 2D) showed even relatively lower ThT values in the tests of back neck and ear than that of arm and thigh. It implies a wide distribution of prion agents in skin tissues of sCJD patients during diseases.

To evaluate the potential influence factors for the reactivity in skin RT-QuIC test, 29 tested patients were grouped based on the reactivities of the skin RT-QuIC (17 positive and 12 negative) and the associations with some main data of clinical, clinical examinations and laboratory were analyzed. As shown in Table 4, despite of slight differences between the skin RT-QuIC positive and negative groups, no statistical significance was addressed on the items of onset age, gender and polymorphisms on codon 129 and 219. PSWC on EEG seemed to be a positive related factor to the positive reaction in skin RT-QuIC, as 6 out of 17 patients with positive in skin RT-QuIC reported PSWC on EEG, while 8 cases who had received EEG examinations but did not record PSWC are all negative in skin RT-QuIC. Special abnormalities on MRI were recorded in 13 out of 16 patients (one case not performed) showing the positive skin RT-QuIC, while also observed in 6 out 10 cases (two cases not performed) in the negative group. CSF 14-3-3 positive was detected in 10 out of 16 cases (one case not performed) in the positive group and 5 out 12 cases in the negative one. All 8 patients with CSF RT-QuIC positive were also skin RT-QuIC positive.

## Discussion

CSF RT-QuIC assay is becoming an ideal method for the premortem diagnosis of sCJD and prion research based on its reliable specificity and high sensitivity[5, 10]. In this study, we tested the feasibility of skin RT-QuIC assay in the diagnosis for probable sCJD together with the paired CSF samples. We found that all skin samples from 12 probable sCJD patients elicited positive reactions in RT-QuIC assays using recombinant hamster PrP90-231 as the substrate, while none of skin samples from 9 non-CJD cases induced positive reaction. Meanwhile, only 5 out of 11 paired CSF samples from those 12 probable sCJD cases showed positive seeding activity. It highlights higher sensitivity of skin RT-QuIC for prion detection in probable sCJD cases than that of CSF RT-QuIC.

Our data here revealed dose-dependent manner of the tested skin homogenates in RT-QuIC tests. The highest positive rate was observed in the reactions of the lowest dilution ( $10^{-2}$  dilution). On contrary, our study and many other studies have demonstrated that the brain tissues of sCJD patients can induce positive reaction in RT-QuIC at very high dilution ( $10^{-8}$  dilution) [14, 15]. Obviously, the reactivity of RT-QuIC depends on the amount of tissue PrP<sup>Sc</sup>. Usually, it is almost impossible to detect PrP<sup>Sc</sup> in the skin specimen of CJD patients with routine techniques, such as protease- or guanidine-treated Western blot and immunohistochemistry, suggesting very low concentration of PrP<sup>Sc</sup> in skin specimen. It is worth noting that two skin samples in this study exhibited positive reactions at relatively higher dilutions ( $10^{-3}$  and  $10^{-4}$ ) but failed to elicit positive reaction at low dilution ( $10^{-2}$ ). The exact reason is unknown at

present. Our previous CSF RT-QuIC tests of human PrDs also revealed similar phenomenon that excessive amounts of CSF from some patients had a low or no reactivity of prion-seeding activity. Possibly, some unknown agents in human tissues may affect the RT-QuIC reactivity. Nevertheless, based on our preliminary data we suggest that it is better to perform skin RT-QuIC assays within a certain range of dilutions, such as  $10^{-2}$  and  $10^{-3}$  of tissue homogenate for the diagnosis of human PrDs.

Skin samples from different body sites were obtained from four patients, including two of posterior neck and lateral malleolus, one of posterior neck and medial forearm, as well as one of behind ear and inner thigh, respectively. All of them induced positive RT-QuIC reactions, indicating widely distribution of prion agents in skin tissues during the clinical courses. According to our results, the RT-QuIC reactions of the skin samples from the posterior neck seem to be stronger than that of distal extremities. This result seems to be coincidental with the observations described by Zou and his colleagues, that the skin specimens of sCJD patient around the head and ear showed the highest sensitivity in RT-QuIC test [11]. However, the other two cases with the skin samples from head and neck did not illustrate strong reaction in RT-QuIC compared with their individual samples from arm and thigh. Further study with more cases is required to draw the final conclusion.

Five patients in this study were grouped as the pending cases when the skin RT-QuIC assays were conducted. Four of them were positive in skin RT-QuIC tests. Dementia was recorded in all five cases. However, the other clinical symptoms still did not meet the sCJD diagnostic criteria. Up to the preparation of this report, there is no final diagnosis being made for those five patients. The careful follow-up of the five patients are needed not only for the clinical diagnosis, but also for the evaluation of diagnostic significance of skin RT-QuIC in the patients who are suspected as sCJD.

The skin samples from three different gPrD cases were also included in this study. Only the case of T188K gCJD showed positive in skin RT-QuIC test. T188K gCJD is the second most frequently observed gCJD type in Chinese [16]. More importantly, T188K gCJD is obviously predominant among Chinese patients, but rarely described in other countries, even in Japan and Korea [17]. Our previous study has revealed that 52% (13/25) T188K gCJD cases are positive in CSF RT-QuIC [13]. Coincidentally, the T188K gCJD case in this study was also positive in CSF RT-QuIC. D178N FFI is the most frequently detected gPrD in Chinese [16]. The clinical phenotype and neurological abnormality of D178N FFI are markedly different from GSS and other gCJDs [18]. Our study has verified a remarkably low positive rate and weak reactivity in CSF RT-QuIC of FFI patients compared with the cases of E200K and T188K gCJD [13]. It is not surprising that the FFI case here was negative in skin RT-QuIC. G114V gCJD is rare subtype among Chinese and only 4 cases have been identified in our surveillance system since 2006 [16]. Beside of the case here, another G114V gCJD Chinese case showed negative in CSF RT-QuIC as well (data not shown). Due to the diversity of human gPrDs, the diagnostic significance of skin RT-QuIC needs further analysis.

Reviewing of some clinical features of the tested patients with sCJD did not show any significant correlation with the results of skin RT-QuIC. Some sCJD associated examination and laboratory tests seemed to be statistically correlated with the reactivity in skin RT-QuIC, such as PSWC on EEG, implying

its accuracy for the diagnosis of sCJD. Therefore, it is most likely that those skin RT-QuIC associated factors probably reflect the close disease-related phenomenon.

## Conclusions

The skin RT-QuIC assay is still at the starting stage. Our preliminary data revealed it high sensitivity in probable sCJD patients and reliable specificity in non-CJD cases, which provides proof of concept that skin RT-QuIC is a suitable tool for premortem diagnosis of sCJD.

## Abbreviations

cerebrospinal fluid	CSF
diffusion-weighted imaging	DWI
fluorescence units	rfu
genetic prion disease	gPrD
iatrogenic CJD	iCJD
Periodic sharp wave complexes	PSWC
sporadic Creutzfeldt-Jakob disease	sCJD
variant CJD	vCJD

## Declarations

### *Ethics approval*

Usages of the CSF and skin samples and relevant clinical information of the patients with different diseases in the Center of Chinese CJD Surveillance System has been approved by the Ethics Committee of the National Institute for Viral Disease Control and Prevention, China CDC, under the protocol of 2009ZX10004-101.

### *Consent for publication*

Not applicable

### *Availability of data and materials*

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### *Competing Interests*

The authors report no competing interests.

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### ***Authors' contributions***

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Kang Xiao, Xue-Hua Yang and Wei Zhou. The first draft of the manuscript was written by Kang Xiao and all authors commented on previous versions of the manuscript.

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Not applicable

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## Tables

**Table 1. The demographic information of 15 cases from US CJD surveillance center**

Case No.	Onset age	Gender	Diagnosis	Skin RT-QuIC
Case 1	68	F	Non-CJD	-
Case 2	78	M	Non-CJD	-
Case 3	84	F	Non-CJD	-
Case 4	65	M	Non-CJD	-
Case 5	58	M	Non-CJD	-
Case 6	68	M	sCJD MV1-2	+
Case 7	59	F	sCJD MM1	+
Case 8	65	F	sCJD MM1-2	+
Case 9	67	M	sCJD MM1	+
Case 10	79	M	sCJD MV2	+
Case 11	60	F	sCJD MV1-2	+
Case 12	57	F	sCJD MM1	+
Case 13	71	F	sCJD VV2	+
Case 14	78	F	sCJD MM1	+
Case 15	68	F	sCJD MM1	+

**Table 2. The main clinical and laboratory information of the patients with different diseases from Chinese CJD surveillance system**

Diagnosis	Onset age	Gender	Codon 129	Codon 219	EEG	MRI	CSF 14-3-3	CSF RT-QuIC	Skin RT-QuIC	Skin sampling site
Probable sCJD										
Case 1	72	F	MM	EE	-	+	-	-	+	abdomen
Case 2	70	M	MM	EE	-	-	+	-	+	abdomen
Case 3	69	F	NA	NA	+	+	+	-	+	inner thigh
Case 4	64	F	MM	EE	-	NA	+	+	+	medial upper arm
Case 5	68	M	MM	EE	+	+	-	+	+	posterior neck
Case 6	66	M	MM	EE	-	+	-	+	+	medial upper arm
Case 7	62	M	MV	EE	-	-	+	+	+	medial upper arm
Case 8	66	F	MM	EE	+	-	+	-	+	inner thigh
Case 9	69	F	MM	EE	+	+	-	-	+	posterior neck, lateral malleolus
Case 10	65	F	MM	EE	+	+	NA	NA	+	medial upper arm
Case 11	54	M	MM	EE	-	+	+	+	+	medial upper arm
Case 12	84	M	MM	EE	-	+	+	-	+	medial forearm, back neck
Genetic PrD										
T188K gCJD	60	F	MM	EE	+	+	-	+	+	behind ear
G114V gCJD	38	M	MV	EE	-	+	+	-	-	behind ear

D178N FFI	66	F	MM	EE	-	-	-	-	-	medial upper arm
Pending case										
Case 1	53	F	MM	EE	-	-	+	-	-	medial upper arm
Case 2	63	F	MM	EE	-	+	-	-	+	chest
Case 3	44	F	MM	EE	-	+	+	-	+	abdomen
Case 4	55	F	MM	EE	-	+	+	+	+	behind ear, inner thigh
Case 5	58	F	MM	EE	-	+	+	+	+	posterior neck, lateral malleolus
Non-CJD										
Case 1	43	F	MM	EE	NA	+	-	-	-	abdomen
Case 2	52	M	MM	EE	NA	+	-	-	-	behind ear
Case 3	65	M	MM	EE	-	-	-	-	-	behind ear
Case 4	68	M	MM	EK	NA	NA	-	-	-	medial upper arm
Case 5	65	M	MM	EK	-	-	-	-	-	behind ear
Case 6	83	F	NA	NA	-	NA	+	-	-	medial upper arm
Case 7	57	F	MM	EE	-	+	+	-	-	inner thigh
Case 8	64	M	MM	EE	NA	+	-	-	-	medial upper arm
Case 9	66	M	MM	EE	-	+	+	-	-	abdomen

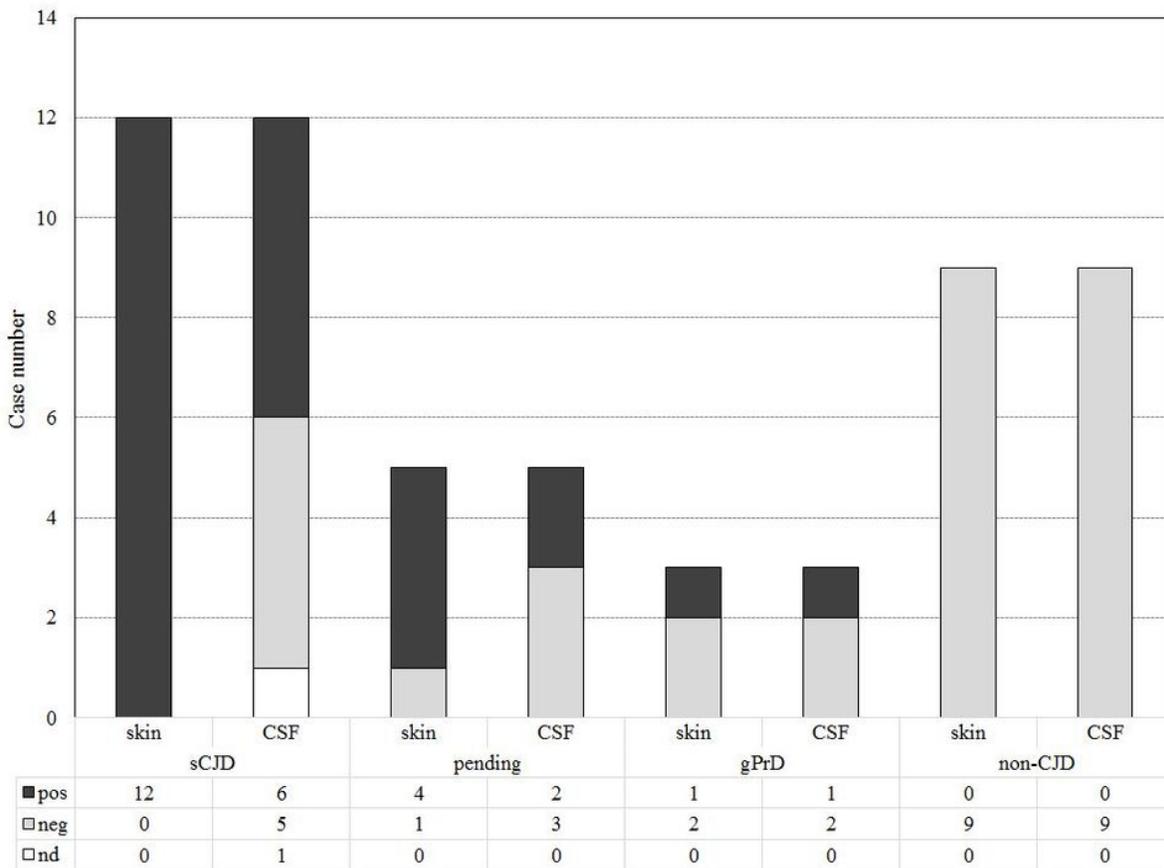
**Table 3. The RT-QuIC reactivity per well of the skin samples at the different dilutions from the patients with various diseases**

Diagnosis	Skin sampling site	Dilution			Final result
		10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	
Probable sCJD					
Case 1	abdomen	-/-/+	-/+/+	-/-/+	+
Case 2	abdomen	+/+/+	-/+/+	-/-/-	+
Case 3	inner thigh	-/-/-	-/-/+	+/+/+	+
Case 4	medial upper arm	+/+/+	-/-/-	-/-/-	+
Case 5	back neck	+/+/+	+/-/-	-/-/-	+
Case 6	medial upper arm	+/+/+	+/-/-	-/-/-	+
Case 7	medial upper arm	+/+/+/+	+/+/+/+	-/-/-/-	+
Case 8	inner thigh	+/+/+/+	+/+/+/+	+/-/-/-	+
Case 9	back neck	+/+/+/+	+/+/+/-	+/+/+/-	+
	lateral malleolus	+/+/+/+	-/-/-/-	-/-/-/-	+
Case 10	medial upper arm	+/+/+/+	-/-/-/-	-/-/-/-	+
Case 11	medial upper arm	+/+/+/+	+/+/+/-	-/-/-/-	+
Case 12	medial forearm	+/+/+/+	+/+/+/-	-/-/-/-	+
	back neck	+/+/+/+	+/+/-/-	+/-/-/-	+
gPrD					
T188K gCJD	behind ear	+/+/+/+	+/+/+/+	-/-/-/-	+
Pending					
Case 2	chest	+/+/+/+	-/-/-/-	-/-/-/-	+
Case 3	abdomen	+/+/+/+	+/+/+/+	-/-/-/-	+
Case 4	behind ear	+/+/+/-	+/-/-/-	-/-/-/-	+
	inner thigh	+/+/+/+	+/+/-/-	-/-/-/-	+
Case 5	back neck	+/+/+/+	+/+/+/+	+/+/-/-	+
	lateral malleolus	+/+/+/+	+/+/-/-	-/-/-/-	+
Total (pos/total, %)	/	19/21(90.5%)	14/21(66.7%)	3/21(14.3%)	/

**Table 4. Comparisons of clinical and laboratory data between skin RT-QuIC positive and negative groups.**

	positive	negative	p-value
Age (median, min-max) (y)	65(44-84)	64.5(38-83)	0.27
Gender (M/F)	6/11	7/5	0.23
Codon 129 (MM/MV)	15/1	10/1	0.79
Codon 219 (EE/EK)	16/0	9/2	0.08
EEG (+/-)	6/11	8/0	0.06
MRI (+/-)	13/3	6/4	0.24
CSF 14-3-3 (+/-)	10/6	5/7	0.28

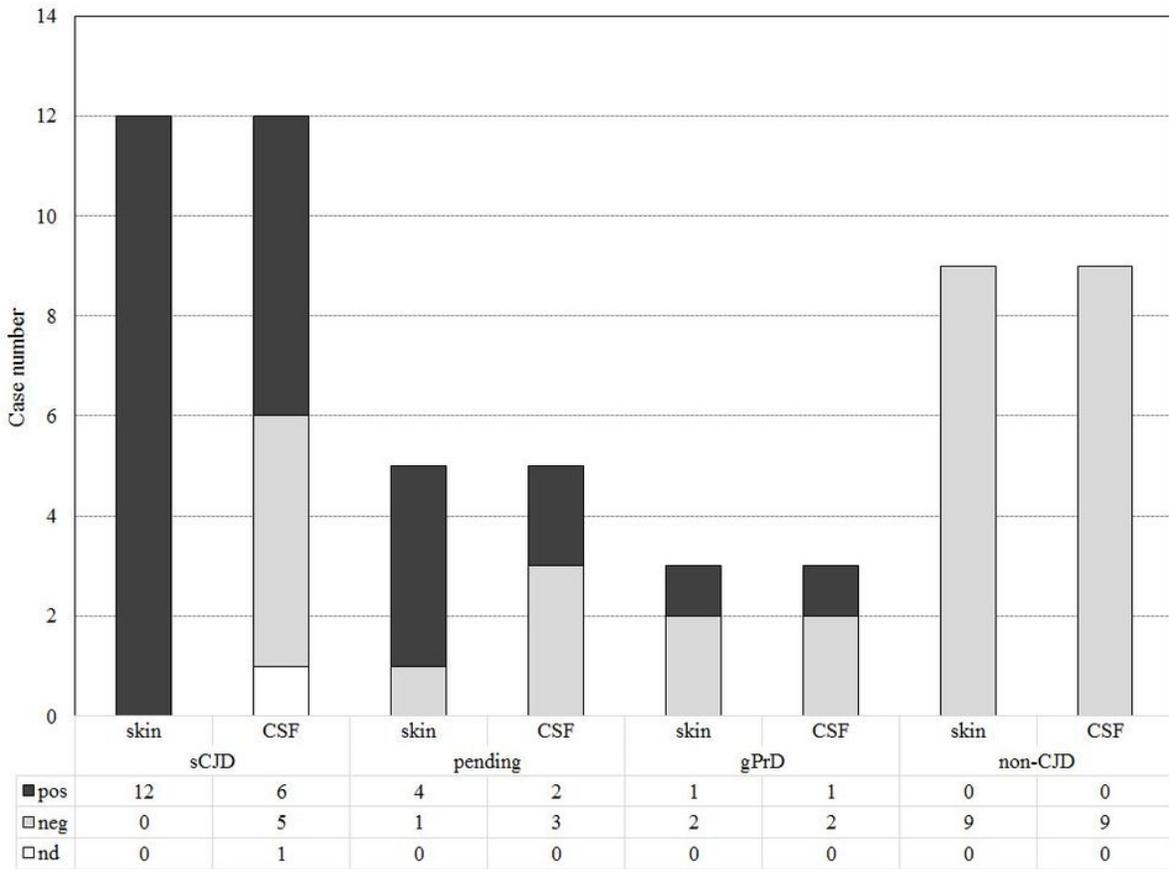
## Figures



Xiao et al, Fig 1

Figure 1

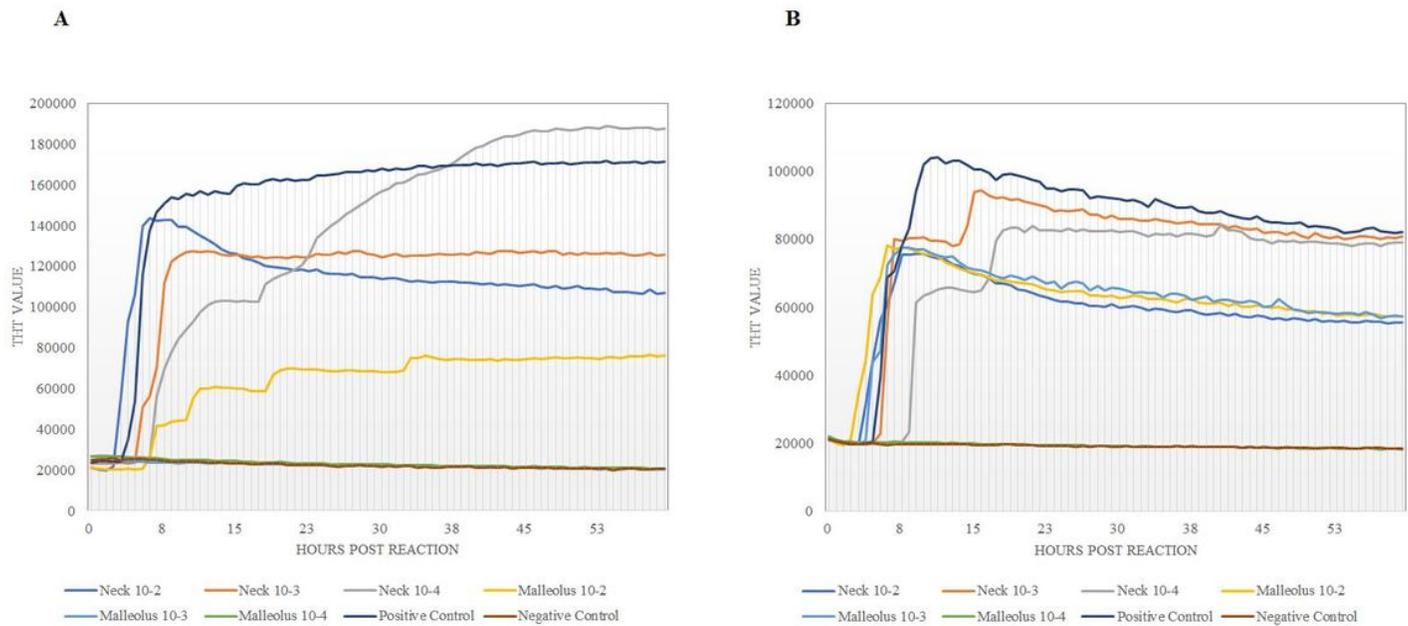
The positive and negative numbers in the skin and CSF RT-QuIC assays among the patients with different diagnosis.



Xiao et al, Fig 1

### Figure 1

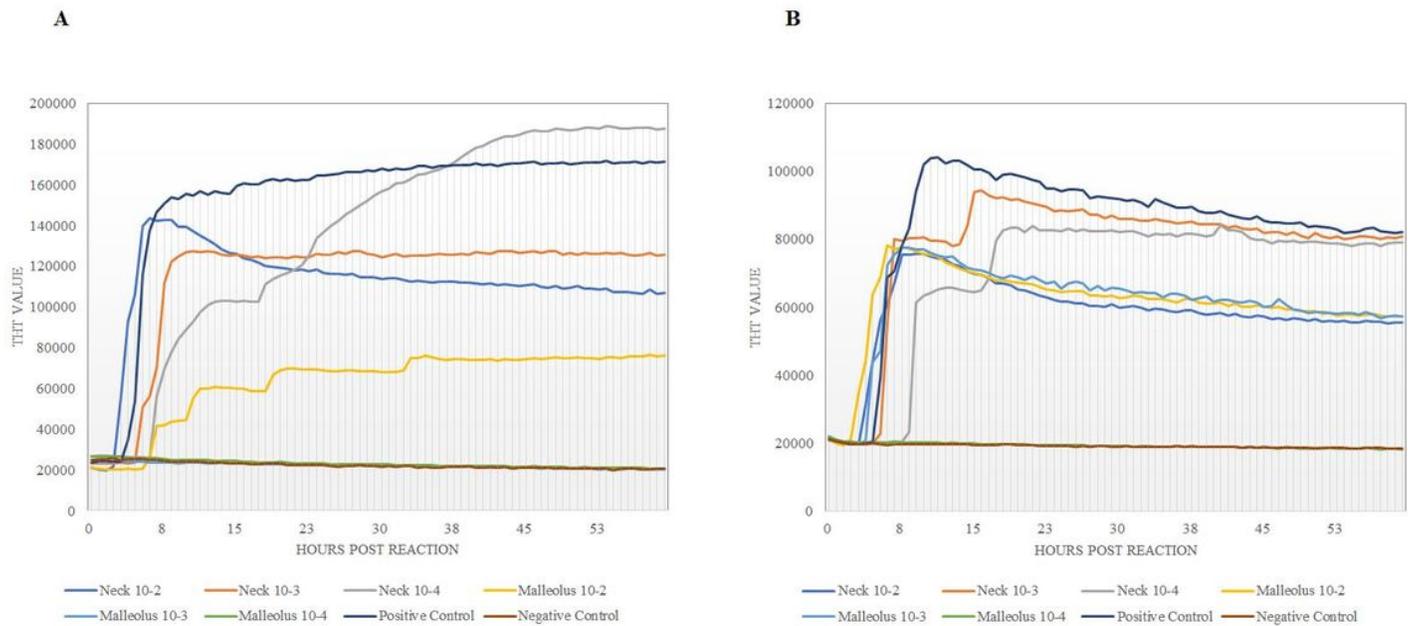
The positive and negative numbers in the skin and CSF RT-QuIC assays among the patients with different diagnosis.



Xiao et al, Fig 2

## Figure 2

Results of RT-QuIC assays of different sites from the same patient (A. sCJD Case 9; B. pending Case 5; C. pending Case 4; D. sCJD Case 12). The skin homogenates in the assays were 10-2, 10-3 and 10-4 diluted. The brain homogenate of scrapie infected hamster was used as positive control and that of normal hamster was used as negative control. ThT value is showed in Y-axis and hour post-reaction is indicated in X-axis.



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